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APPLICATION OF $^{13}C/^{12}C$ RATIOS IN DETECTING ADULTERATION OF APPLE JUICE, ORANGE JUICE, HONEY, AND OTHER FOOD PRODUCTS

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HONEY

As a result of the development of the low cost sweetner, high fructose corn syrup (HFCS), we initiated in 1975 a research program to develop methods to detect the addition of this sweetener to honey. Honey has traditionally been a target for adulteration, and earlier methods were developed[1] to detect addition of conventional corn syrup and commercial invert syrups. The honey industry was concerned that HFCS would be used to adulterate honey, and because its sugar composition is in the range normally found for honey, and refining leaves little else, the existing methods were unsuitable for its detection.

Many approaches were taken, with the hope that a constituent or physical property could be identified which is unique to HFCS, and not present in any of the wide varieties of honey. The various approaches have been reviewed elsewhere,[2] and three were found to be useful for detecting the undeclared addition of HFCS to honey. These are a thin-layer chromatographic (TLC) procedure,[3] which revealed high molecular weight oligosaccharides that are unique to HFCS; a gas-liquid chromatographic (GLC) procedure,[4] which demonstrated that HFCS has a greater ratio of the disaccharides isomaltose:maltose; and a stable carbon isotope ratio analysis (SCIRA) method, which showed HFCS to have higher $^{13}C/^{12}C$ ratios than honey. The SCIRA and TLC procedures are official first action Association of Official Analytical Chemists (AOAC) methods, and are widely used.

STABLE CARBON ISOTOPE RATIO ANALYSIS

The $^{13}C/^{12}C$ mass spectrometric method is applicable because this ratio in an organic material reflects the pathway of photosynthesis in the source plant. Plants using the Calvin (C_3) cycle possess $\delta^{13}C$ values of -22 to -33 ppt (parts per thousand) and those using the Hatch-Slack (C_4) cycle have $\delta^{13}C$ values ranging from -10 to -20 ppt.[5,6] Corn, sorghum, and sugar cane are Hatch-Slack plants, and the organic materials derived from them, such as sugars, are enriched in ^{13}C compared with Calvin plants. So samples with higher $\delta^{13}C$ values (i.e., less negative) are richer in ^{13}C than those with lower (more negative) $\delta^{13}C$ values, since:

$$\delta^{13}C\,(\text{ppt}) = \frac{(^{13}C/^{12}C)\ \text{sample}}{(^{13}C/^{12}C)\ \text{standard}} - 1 \times 10^3$$

These values are determined after complete combustion of the sample to carbon dioxide and water, and comparing the ratios of $^{13}C^{16}O_2$ and $^{12}C^{16}O_2$ with a precise isotope ratio mass spectrometer.

As expected, HFCS was found to average -9.7 ppt in $\delta^{13}C$, characteristic of a material derived from a Hatch-Slack plant. If all honeys could be shown to have values characteristic of Calvin plants, an effective method to detect honey-HFCS mixtures could be developed. Since honeybees make honey from the nectar of a wide variety of flowering plants, it was necessary to analyze many samples of known floral source for $\delta^{13}C$. Domestic (84 samples) and imported (35 samples) honeys were tested, and the results are shown in Table 1.[7] The coefficient of variation was just 3.86%, the smallest yet encountered for any honey constituent or physical property. Of the eighty-four domestic honey samples tested, 34 states and 37 floral sources from 17

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plant families were represented, including the commercially most significant. Table 2 summarizes the results for at least one sample from each family, and Figure 1 shows the distribution of $\delta^{13}C$ values for all honey samples and four HFCS samples. Knowing that HFCS averages -9.7 ppt and honey -25.4 ppt in $\delta^{13}C$, mixtures were prepared and tested. It was shown that these mixtures possessed $\delta^{13}C$ values equal to the sum of the fractional contribution of each material. Without knowing the value of the honey from which a suspected mixture is prepared, it is not possible to assign % HFCS values to such mixtures. Instead, a statistical approach must be used based on the standard deviation for all honeys.

COLLABORATIVE STUDY-HONEY

In order to obtain AOAC official method sanction for using the $^{13}C/^{12}C$ method to detect honey adulteration, the following procedure was followed. Five samples were sent to each of seven laboratories. Four samples contained from 25-26% honey; the remaining sample was pure honey. The collaborating laboratories did not know the composition of the samples, and were asked to analyze each for $\delta^{13}C$. The results of the collaborative study are given in Table 3, where it can be seen that good agreement was demonstrated. Also, the sample which contained HFCS had appropriate $\delta^{13}C$ values. As a result, this is now an official method. How the method is being used by the honey industry and the regulatory agencies will be described later, along with the results regarding apple and orange juices.

APPLE JUICE

Concern was expressed in 1979 by the apple processing industry that HFCS may be used for adulterating apple juice and apple juice concentrates. In the same year, newspapers reported [9,10] results of tests they conducted on market samples of apple juice. The results, obtained by $^{13}C/^{12}C$ analysis, indicated that several juices were incorrectly labelled as pure juice.

We undertook to demonstrate that juices from the commercially significant apple varieties possessed the expected range of $\delta^{13}C$ values, as they are Hatch-Slack plant derived materials.[11] As shown in Table 4, the range for 41 pure apple juices was -22.5 to -27.9 ppt, similar to the range earlier reported for honey samples, but with a slightly greater coefficient of variation. The individual $\delta^{13}C$ values for 40 samples are given in Table 5, and the distribution of values is shown in Figure 2.

COLLABORATIVE STUDY-APPLE JUICE

A collaborative study[12] was then conducted in a manner similar to that described earlier for honey. The collaborators were in good agreement, the samples with HFCS had appropriate $\delta^{13}C$ values, and the results are given in Table 6. The carbon isotope method is official first action AOAC.

ORANGE JUICE

In response to requests from the orange juice industry, work is underway to test the application of $\delta^{13}C$ analysis to orange juice adulteration. Orange juice is from a Calvin plant and has characteristic $\delta^{13}C$ values, as found in an earlier study of Israeli juices.[13] We have tested domestic juices from many locations and found them to be quite uniform in $\delta^{13}C$, with values similar to honey and apple juice. A collaborative study to test the application of $\delta^{13}C$ analysis in detecting adulteration of orange juice with HFCS is underway, with promising preliminary results.

APPLICATION OF THE OFFICIAL SCIRA METHODS

Because of the range in $\delta^{13}C$ values for pure honeys and juices, the method is a qualitative one; it cannot accurately determine the degree to which a sample has been adulterated. This is because one does not know the value of the pure material used for blending. The proper manner in which to interpret $\delta^{13}C$ values for honey adulteration was detailed in a recent report.[14] Since $\delta^{13}C$ values for both pure honeys and apple juices have been found to extend up to -22.5 ppt, suspect samples with ratios more negative than -22.5 ppt cannot be classified as adulterated based solely on this method. A statistical approach is required, based on standard deviations from the mean for all samples, as shown below.

	Mean $\delta^{13}C$	Standard Deviation(Sd)	4Sd
Honey	-25.4	0.980	-21.5 ppt
Apple Juice	-25.3	1.275	-20.2 ppt

At the 4Sd limit there is only one chance in 25,000 that the sample is authentic. The probability decreases with increasing (less negative) $\delta^{13}C$ values.

For interpreting honey results, it has been recommended[14] that "samples with $\delta^{13}C$ values between -23.4 and -21.5 ppt are considered adulterated only if a positive result is also obtained from the TLC method."

The honey, apple juice industries and regulatory agencies are actively policing adulteration practices so that they will be discouraged and hopefully eliminated so that the integrity of these markets be maintained.

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Figure 1: Distribution of $\delta^{13}C$ values among 4 HFCSs and 119 samples of honey including 4 honeydew honeys.

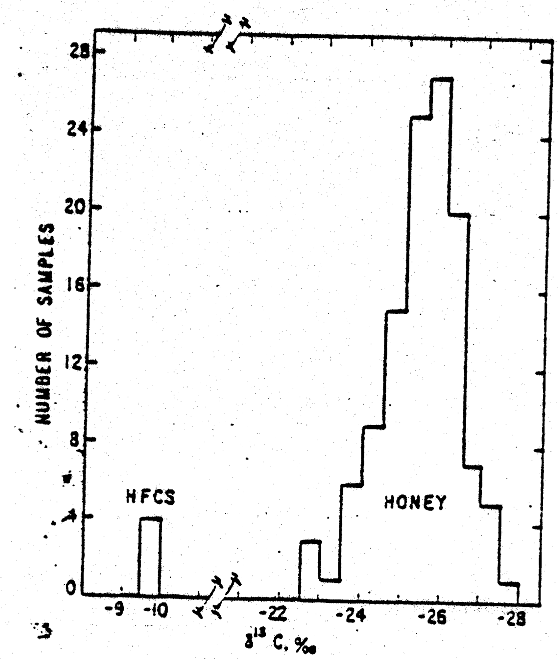

Figure 2: Distribution of $\delta^{13}C$ (ppt) values among apple juices.

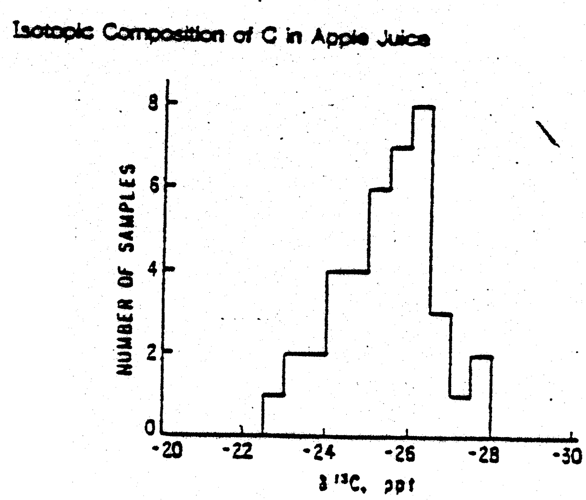

Table 1: $\delta_{13}C$ of honeys and HFCSs[a]

Source	No. of samples	Mean, °/oo	Range High	Range Low	Std dev., °/oo	Coeff. of var., %
United States honey	84	-25.2	-22.5	-27.4	0.94	3.73
Imported honey	35	-25.8	-23.9	-27.4	0.97	3.76
All honey	119	-25.4	-22.5	-27.4	0.98	3.86
HFCS	4	-9.7	-9.5	-9.8	0.14	1.4

Table 2: SCIRA Values of Selected U.S. Honeys

Family	Floral type	Number of samples	$\delta_{13}C$ (per mil)
Anacardiaceae	Schinus molle (pepper tree)	1	-25.0
Aquifoliaceae	Ilex glabra (gailberry)	1	-25.6
Compositae	Centauria solstitialis (star thistle)	1	-26.3
Cornaceae	Nyssa ogeche (tupelo)	1	-26.0
Cyrillaceae	Cyrilla parvifolia (titi)	1	-24.2
Euphorbiaceae	Sapium sebiferum (tallow tree)	1	-26.4
Labiatae	Salvia spp. (sage)	1	-24.2
Leguminosae	Glycine soja (soybean)	2	-26.8
Leguminosae	Medicago sativa (alfalfa)	10	-25.2
Leguminosae	Melilotus spp. (sweet clover)	2	-26.4
Leguminosae	Trifolium spp. (clover)	11	-25.6
Magnoliaceae	Liriodendron tulipifera (tulip tree)	1	-25.3
Malvaceae	Gossypium hirsutum (cotton)	1	-24.7
Onagraceae	Epilobium angustifolium (fireweed)	1	-25.4
Palmae	Sabai spp. (palmetto)	1	-24.7
Polygonaceae	Fagopyrum exculentum (buckwheat)	1	-25.2
Rosaceae	Rubus spp. (blackberry)	1	-26.1
Rutaceae	Citrus spp. (orange, grapefruit)	3	-23.4
Tamaricaceae	Tamarix gallica (tamarisk)	1	-25.1
Tiliaceae	Tilia americana (basswood)	1	-25.6
	Unclassified natural season blends	15	-25.2
	Honeydew honey	4	-24.5

Table 3: $\delta^{13}C$(°/oo vs. PDB) values for collaborative samples

Coll.	Sample A	B	C	D	E
1a	-17.6	-14.0	-19.3	-15.2	-25.1
1b	-17.4	-13.5	-19.5	-14.8	-24.8
2	-17.4	-13.8	-19.5	-15.1	-25.1
3	-17.7	-13.9	-19.8	-14.7	-24.4
4	-17.7	-13.6	-19.3	-15.0	-24.9
5	-18.5	-14.9	-21.4	-16.0	-25.9
6	-17.1	-13.4	-19.3	-14.6	-24.9
Mean	-17.63	-13.87	-19.72	-15.06	-25.01

Table 4: $\delta^{13}C$(ppt) for Apple Juices

Variety	no. of samples	$\delta^{13}C$(ppt) range	$\delta^{13}C$(ppt) mean	SD	CV, %
Cortland	1		-26.1		
Davey	1		-23.9		
Golden Delicious	8	-23.9--26.2	-25.0	0.818	3.27
Granny Smith	1		-23.0		
Green Gravin	1		-26.7		
Idared	1		-27.8		
Macoun	1		-25.1		
MacIntosh	11	-23.2--27.9	-25.8	1.255	4.86
Newtown	1		-24.4		
Red Delicious	4	-22.5--26.2	-24.8	1.640	6.61
Rhode Island Greening	1		-25.7		
Rome	2	-24.2--27.3	-25.8		
Standard	1		-24.0		
Stark Splendor	1		-25.5		
Stayman	1		-26.2		
Winesap	1		-26.0		
Winter Banana	2	-24.6--26.4	-25.5		
York	1		-25.7		
by state					
New York	13	-23.2--27.9	-25.8	1.200	4.65
Virginia	7	-25.1--27.3	-26.0	0.678	2.61
Washington	6	-24.0--26.4	-24.9	1.107	4.45
all samples	40	-22.5--27.9	-25.4	1.239	4.88

Table 5: $\delta^{13}C$ Values of apple juices and HFCSs

Source	No. of samples	Mean,°/oo	Range Low	Range High	Std dev.,°/oo	Coeff. of var.,%
Apple juice	41	-25.3	-27.9	-22.5	1.275	5.04
HFCS	4	-9.7	-9.8	-9.5	0.14	1.4

Table 6: $\delta^{13}C$(°/oo vs. PDB) for collaborative samples

Coll.	Sample A	B	C	D	E
1	-17.8	-23.4	-14.0	-16.1	-19.7
2	-17.0	-22.9	-13.2	-15.1	-18.9
3	-17.4	-23.0	-13.2	-15.4	-19.2
4	-17.5	-20.8	-16.9	-16.0	-19.5
5	-17.7	-22.5	-13.8	-15.5	-19.4
6	-16.9	-22.9	-13.1	-15.0	-19.0
Mean	-17.4	-22.6	-14.0	-15.5	-19.3

A B S T R A C T
for
List of Publications

: APPLICATION OF $^{13}C/^{12}C$ RATIOS IN DETECTING ADULTERATION OF APPLE
: JUICE, ORANGE JUICE, HONEY AND OTHER FOOD PRODUCTS.
: by Landis W. Doner
: "Proceedings of Symposium on Technological Problems of Fruit Juice
: Concentrate"

Honey, apple juice and orange juice have been targets for adulteration with high fructose corn syrup (HFCS) and methods have been needed to detect such mixtures. Mass spectrometric determination of $^{13}C/^{12}C$ ratios has been demonstrated to be an effective and non-circumventible approach. The method is based on the fact that organic materials derived from plants using the Calvin photosynthetic cycle, such as all honey floral sources, and orange and apple trees are measurably depleted in ^{13}C compared to HFCS from corn, a Hatch-Slack photosynthetic plant. Numerous varieties of honey and apple and orange juices have been tested, and $^{13}C/^{12}C$ ratios are uniform. This has provided the basis for the development of official methods for detecting HFCS adulteration of honey and apple juice, and preliminary results suggest that the approach will be extended to orange juice.

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